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Immune complex induced glomerular lesions in C5 sufficient and deficient mice

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Immune complex induced glomerular lesions in C5 sufficient and deficient mice. The role in the pathogenesis of immune complex–mediated glomerulonephritis of C5 or some terminal complement component dependent upon C5 for activation was explored in a congenic strain of C5 sufficient (NSN) and C5 deficient (OSN) mice. When these mice were given daily injections of heterologous protein, horse apoferritin (HAF), there were profound differences between the strains in the development of glomerulonephritis and renal dysfunction. When NSN and OSN mice produced low levels of anti-HAF, NSN mice developed extensive glomerular deposits of HAF and immune reactants and a mild proliferative glomerulonephritis. In contrast, comparable OSN mice developed only trace mesangial localization of HAF and no glomerular lesions by light microscopy. When NSN and OSN mice produced high levels of anti-HAF, both strains had equivalent glomerular immune deposits; however, NSN mice developed a severe necrotizing and crescentic glomerulonephritis, while OSN mice had much less glomerular injury. Compared to OSN mice, these NSN mice also had much more severe tubulointerstitial injury, and significantly higher serum creatinine levels. Thus, in this experimental model, the absence of C5 resulted in reduced glomerular immune complex localization when there were small amounts of circulating immune reactants; and in markedly reduced glomerular leukocyte influx, necrosis and crescent formation, when large amounts of immune reactants have localized in glomeruli. These effects could be mediated by C5 (such as C5a) or by some terminal complement component(s) dependent upon C5 for activation.

Complement is a major mediator of the renal injury and dysfunction that results from immune complex localization in glomeruli, either by deposition from the circulation or by in situ formation. Complement is similarly involved with mediating immune complex–induced injury in other tissues. In both humans and experimental animals with immune complex–induced renal diseases, classical pathway, alternative pathway, and terminal complement components are often demonstrable within glomerular immune deposits (1–3). The pathogenetic role of complement has been investigated in a number of experimental models of immune–mediated glomerular disease. In nephrotoxic nephritis of rats, depletion of plasma complement components with cobra venom factor prevents development of the renal injury, due at least in part to abrogated leukocyte chemotaxis [4, 5]. In a rat model of membranous glomerulopathy, complement depletion with cobra venom factor has demonstrated a role for complement activation in inducing proteinuria [6]. In a similar model, rabbits with normal complement developed proteinuria, but C6-deficient rabbits did not, indicating an effect of the terminal complement components in mediating glomerular injury [7]. These models of membranous glomerulopathy do not appear to involve leukocytes in their pathogenesis. In contrast, when rabbits were given a single dose of antigen and developed immune complex nephritis, complement depletion did not diminish glomerular injury [8]. Our studies were designed to investigate the role of C5 or some terminal complement component dependent on C5 for activation in the pathogenesis of murine immune complex–mediated proliferative glomerulonephritis of varying severity, ranging from mild mesangiopathic changes to severe crescentic glomerulonephritis with extensive leukocyte infiltration.

Glomerulonephritis was induced by the administration of a heterologous protein, horse apoferritin (HAF) [9, 10] in a congenic mouse strain differing in a single gene locus coding for the presence or absence of C5. This experimental model has advantages over previously studied models in that: 1) the problem of variation in strain between experimental and control animals encountered in C6-deficient rabbit studies is avoided by using congenic animals; 2) the formation of activated complement components inherent in the use of cobra venom factor is prevented; and 3) the leukotactic and/or membranolytic role of C5 can be evaluated in glomerular lesions of varying severity, including severe proliferative and crescentic glomerulonephritis. These studies document a critical role for C5, or some terminal complement component(s) dependent upon C5 for activation, in the development of immune complex–induced glomerular inflammation and renal dysfunction.

Methods

B.10.D2 NSN (C5 sufficient) and B.10.D2 OSN (C5 deficient) male mice were obtained from Jax Laboratory (Bar Harbor, Maine, USA). Animals were divided into three experimental groups: Group I animals were five to nine weeks of age at the time of initial antigen administration. They received a high dose of antigen 4 mg of HAF (Sigma Chemical Co., St. Louis, Missouri, USA) in 0.1 ml of physiologic saline injected intraperitoneally (i.p.) six days per week (high dose schedule). Group II mice were four to eight months of age and were given

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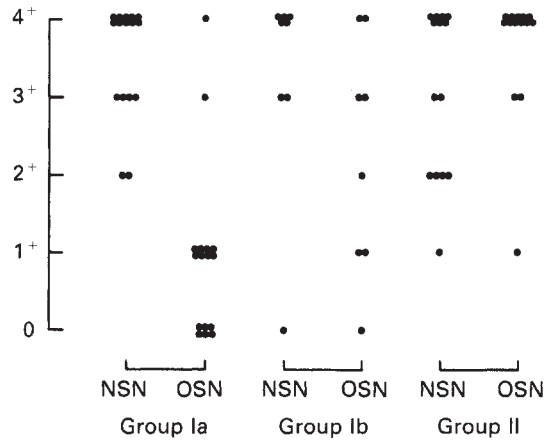

Fig. 1. *Immunofluorescent localization of HAF in glomeruli was graded from 0 to 4+ on the basis of intensity and distribution of the antigen.* There was no difference in HAF localization in NSN and OSN mice in group Ib and II who mounted a high-titered antibody response at four weeks. In Group Ia, NSN mice had significantly more HAF in their glomeruli than similarly treated OSN animals ($P < 0.001$).

the same high dose of HAF. Group III mice were five months old and received a low dose schedule of 1 mg HAF given i.p. every other day. Injections were continued for two, four, and six weeks, and the animals sacrificed at these predetermined intervals. Cage control mice in both five to nine week and four to eight month animals were sacrificed for study at the six week time point. Whenever possible, plasma was obtained from the retroorbital plexus under ether anesthesia in heparinized Natelson blood collecting tubes (Fisher Scientific, Pittsburgh, Pennsylvania, USA). The plasma was separated and stored at −70° until used. Animals were sacrificed by cervical dislocation, the kidneys removed, and processed for light, immunofluorescence, and electron microscopy. For immunofluorescence microscopy, blocks of tissue were snap frozen in liquid nitrogen, embedded in OCT and stored at −70° until sectioned. Four micrometer sections were stained with fluoresceinated goat antibodies to mouse IgG, IgM and C3, and with a rhodamine-labelled goat anti-HAF (Cappel Laboratories, Inc., Cochranville, Pennsylvania, USA). Preparations were examined using a Leitz Orthoplan microscope equipped for incident light fluorescence microscopy (Leitz, Heerbrugg, Switzerland). The location of immune deposits (that is, mesangial or capillary wall) and intensity (0 to 4+) of immunostaining were determined. Tissues for light microscopy were fixed in B-5 solution, and paraffin sections stained with hematoxylin and eosin. Glomerular morphologic lesions were scored blindly by both observers on the basis of the following parameters: 0—no glomerular lesion; 1+—a segmental hypercellular lesion in many but not all glomeruli; 2+—extensive hypercellularity throughout all glomeruli; 3+—a necrotizing lesion defined by the presence of karyorrhexis; 4+—crescentic nephritis. In all cases mice with necrotizing lesions also had 2+ hypercellularity, and those with crescentic disease had necrosis and hypercellularity. Glomerular cell counts were performed on all tissues. At least five cortical glomeruli sectioned through the hilus were selected randomly, the number of nuclei counted and a mean number of cells per glomerulus determined for each animal. Results are expressed as the group mean which is the mean of the mean number of glomerular cells for each animal.

Samples for electron microscopy were fixed in glutaraldehyde, followed by post-fixation in osmium tetroxide, and embedded in Polybed 812 as previously described [9, 10]. Sections were doubly stained with uranyl acetate and lead citrate. Ultrastructural examination was carried out with a JEOL JEM-T7 transmission electron microscope (Jeol Ltd., Tokyo, Japan). Special attention was paid to the quantity and location of glomerular electron-dense deposits.

Quantitative assay for plasma anti-HAF

An ELISA was established to measure plasma antibodies to HAF. Plasma samples obtained at the time of sacrifice were separated and stored at −70°. The ELISA was performed in 96 well microtiter plates (Costar, Cambridge, Massachusetts, USA) based on a previously described method [1]. The wells were coated with 0.1 mg/ml HAF in phosphate buffered saline (PBS) (0.15 M NaCl, 0.01 M NaH_2PO_4, pH 7.4). After one hour incubation at room temperature, then at 4° overnight, the wells were washed thrice with PBS—0.5% Tween—20 (Sigma). Nonspecific binding of protein to the wells was prevented by incubation for one hour with 5% bovine serum albumin (Sigma). After another wash, a standard dilution of plasma was added for one hour at room temperature, the wells were washed as before, and a dilution of peroxidase labelled goat anti-mouse IgG (γ and μ chain specific) (Tago, Inc., Burlingame, California, USA) was added for 20 minutes. The substrate for the reaction was a solution of 0.04% ortho-phenylendiamine dihydrochloride (Eastman Kodak Co, Rochester, New York) in a solution of citrate phosphate (0.5 M citric acid and 0.1 M NaH_2PO_4, pH 5.0) with 0.012% hydrogen peroxide (Fisher Scientific, Pittsburgh, Pennsylvania, USA). The optical density was read at 450 nm at 20 minutes with a Titertek multiscan (Flow Laboratories, Helsinki, Finland). All samples were run in duplicate. Test samples were compared with control mouse plasma and with a standard pool of plasma obtained from hyperimmunized BALB/C mice. Results are expressed as percentage of the value of the hyperimmune pool.

Measurement of hemolytic activity and serum creatinine levels

To identify NSN and OSN mice, a sensitive total hemolytic assay (CH50) was performed. A modification of the standard CH50 [11] assay was required in that sheep red blood cells were sensitized (EA) with a 1:75 dilution of hemolysin, and washed in cold gelatin veronal buffered saline. Mouse serum was harvested from the retroorbital plexus and immediately placed on ice. After separation, the serum was added to EA within 30 minutes of the time that the hemolysin had been added. B.10.D2.OSN sera had negligible hemolytic activity, whereas B.10.D2 NSN sera consistently demonstrated hemolytic activity from 50 to 100% of the complete lysis control sample. Serum creatinine levels were measured using an adaptation of the autoanalyzer method [12].

Statistical analysis

For these data, several comparisons were carried out in order to ascertain whether strain or group differences existed. For

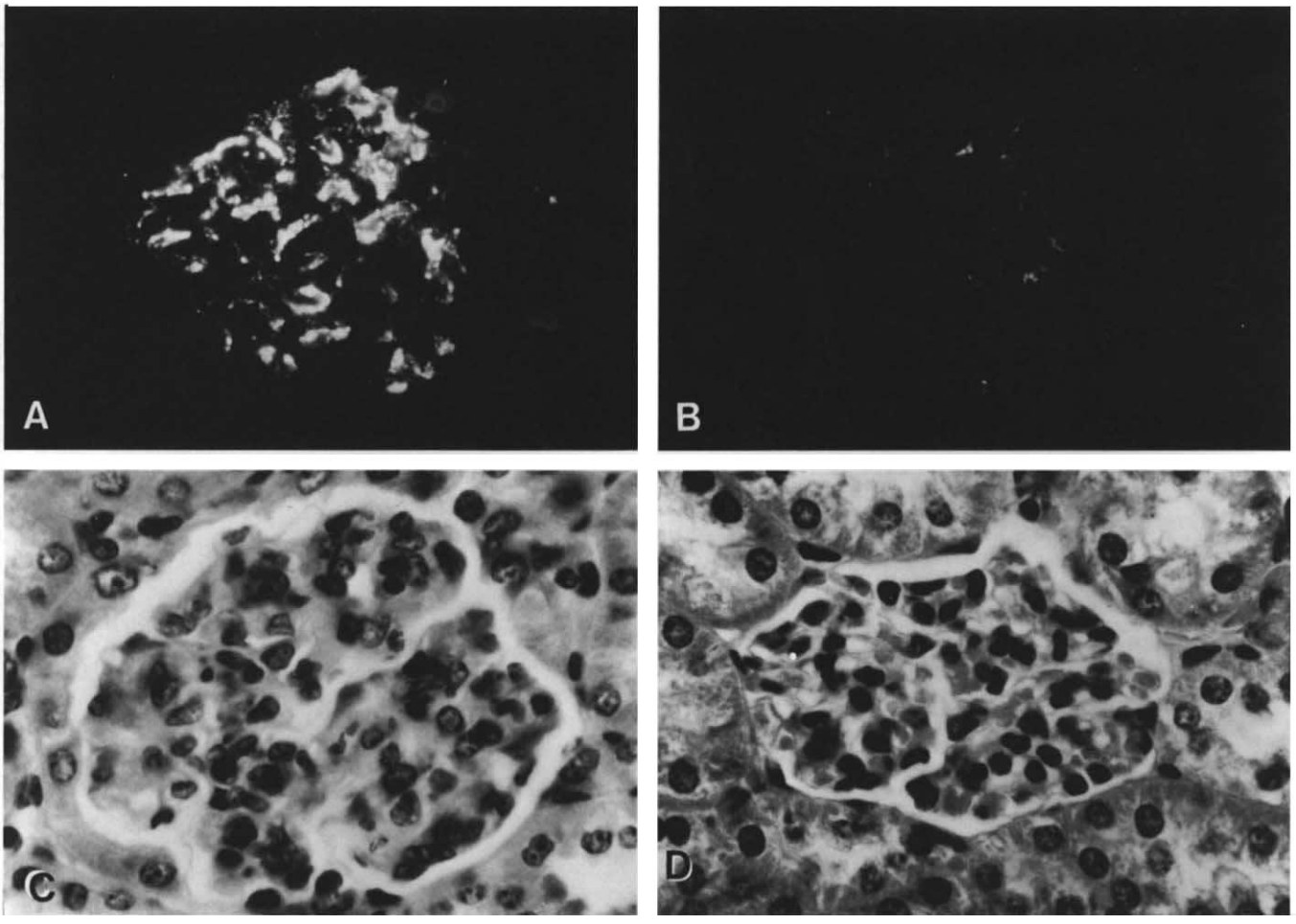

Fig. 2. *Five to nine–week–old NSN mice given 4 mg HAF daily for six weeks who mounted low titer antibody responses (Group Ia) had more staining for HAF in their glomeruli (A) than similarly treated OSN mice (B).* Staining for IgG, IgM and C3 was similarly more intense in NSN than OSN mice. NSN mice in this group had 2+ proliferative mesangial lesions (**C**), whereas OSN mice did not have a morphologic abnormality (**D**). All photomicrographs X600, A and B anti-HAF immunofluorescence microscopy, C and D hematoxylin and eosin.

continuous variables, such as serum creatinine, antibody levels and glomerular cell counts, an analysis of variance was performed to simultaneously analyze the data from all four groups. *T*-tests were then utilized to assess the significance of differences between Groups Ia vs. Ib, vs. II, vs. III and between NSN and OSN mice for any given group. For discrete ordinal variables, such as glomerular lesions, extent of tubulointerstitial infiltrate or of immunofluorescent antigen localization, the non-parametic analog of the *t*-test, the Wilcoxon rank–sum test, was utilized in assessing the significance of strain differences in each group. Because so many tests were performed, a Bonferroni correction was made to control the significance level of the tests. In this correction, the alpha level (0.05 for the tests) is divided by the number of tests being made.

In order to assess the relationship between the morphologic composite score and serum creatinine for Group II, a linear regression model was fit to these data. The significance of the departure from zero of the coefficient of 0.82 was carried out using the F statistic.

The data management and analysis of these data were carried out utilizing the Statistical Analysis System [13].

Results

Group Ia, 5–9 week old mice with low titer antibody production

In two separate experiments, a total of 40 five to nine week old mice were given 4 mg HAF six days per week. The results from these two experiments were similar and the data were pooled.

Four NSN and four OSN animals were sacrificed at two weeks. Immunofluorescence microscopy revealed minimal staining of the tissue by antisera to IgG, IgM, C3 or HAF. Light microscopic examination did not reveal glomerular or interstitial lesions.

Eight NSN and eight OSN animals were sacrificed at four weeks, and the same number of animals at six weeks. No apparent morphologic or immunohistopathologic differences were noted between the four and six week groups, so the

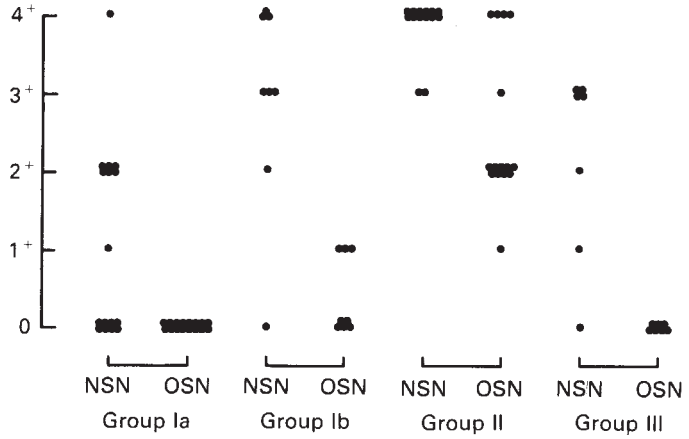

Fig. 3. *Light microscopic morphologic lesions were graded as follows: 0—no lesion; 1+—mild mesangial proliferation in some glomeruli; 2+—proliferative response in all glomeruli; 3+—necrotizing lesions; 4+—crescentic glomerulonephritis.* Five to nine–week–old mice given high dose HAF mounted either a low titered antibody response (Ia) or high titers of anti-HAF (Ib). Group II animals were also given high dose HAF but were four to eight months old; Group III animals were five months old and received 1 mg HAF every other day. There were significant differences between NSN and OSN lesions in Ia, Ib and III ($P < 0.01$) and Group II ($P < 0.001$).

Table 1. Plasma anti-HAF levels determined by ELISA after 2, 4 or 6 weeks of immunization

	NSN	OSN
Group Ia		
2 week	20.5 ± 7.3[a]	17.9 ± 7.8
	$N = 4$	$N = 4$
4 week	22.4 ± 11.4	21.2 ± 7.0
	$N = 8$	$N = 8$
6 week	18.3 ± 6.5	19.3 ± 11.2
	$N = 8$	$N = 8$
Group Ib		
4 week	41.1 ± 8.2	34.8 ± 9.3
	$N = 8$	$N = 8$
Group II		
2 week	57.4 ± 12.5	53.2 ± 20.2
	$N = 4$	$N = 4$
4 week	56.1 ± 26.8	36.2 ± 19.3
	$N = 14$	$N = 14$
Group III		
2 week	78.5 ± 41.2	64.8 ± 0.7
	$N = 5$	$N = 5$
4 week	27.9 ± 20	36.8 ± 20
	$N = 7$	$N = 7$

[a] Expressed as the percentage of the sample value compared with the anti-HAF level in pooled hyperimmunized control animal plasma

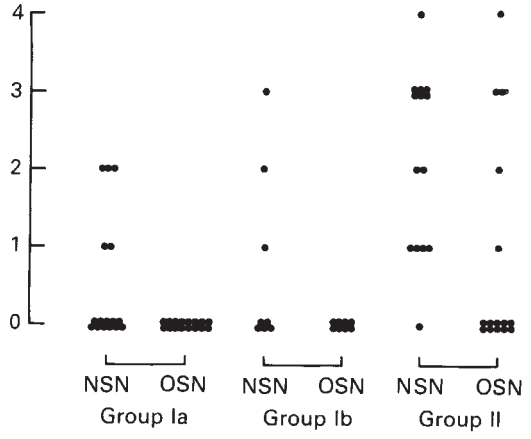

Fig. 4. *Tubulointerstitial lesions were scored on the basis of the percentage of cortex infiltrated with inflammatory cells.* Grading scale is: 0—no apparent tubulointerstitial infiltrates; 1+—less than 25%; 2+—25 to 50%; 3+—50 to 75%; 4+—greater than 75% of cortex involved. While there was no statistically significant difference between the degree of infiltration between NSN and OSN mice in groups Ia and Ib there was a difference between NSN and OSN mice in Group II ($P < 0.01$).

pathologic data from these animals were combined. By immunofluorescence microscopy, 14 of 16 NSN animals had 3 to 4+ mesangial localization of HAF (Figs. 1 and 2). In contrast, 14 of 16 OSN animals had significantly less mesangial HAF deposition ($P < 0.001$). IgG and C3 localization was present with similar intensity and distribution to that of HAF, while IgM was deposited to a lesser extent. In those NSN animals with extensive mesangial immunoreactant localization, granular deposits of HAF, IgG, and C3 were also observed along the capillary walls.

Light microscopic examination of Group Ia did not demonstrate morphologic lesions in the OSN group (Figs. 2D and 3). In comparison, a significant number of NSN animals (seven of 16) ($P < 0.01$) had a 1 to 2+ proliferative glomerular lesion (Figs. 2C and 3), while one NSN mouse had crescentic nephritis. The mean glomerular cell count for NSN mice was 48 ± 2 (mean ± SEM) (cells per glomerulus) ($N = 16$) and was significantly higher than the mean for OSN mice who had 41 ± 2 cells per glomerulus ($N = 16$) ($P < 0.05$). Moreover, five of 16 NSN mice had tubulointerstitial inflammatory cell infiltrates (Fig. 4). On electron microscopy, NSN mice had prominant mesangial as well as occasional subendothelial and subepithelial capillary wall electron dense deposits. OSN mice had only rare mesangial deposits.

Low levels of anti-HAF antibody production were observed in Group Ia mice after HAF injection for two, four and six weeks. At none of these time points was there a difference in anti-HAF levels between NSN and OSN animals ($P > 0.9$) (Table 1). Serum creatinine levels in NSN and OSN mice were equivalent and no higher than control mice (mean of 0.2 mg/dl).

Cage control animals sacrificed at four to six weeks had no detectable antigen deposition in glomeruli and no more than trace immunoglobulin and complement localization. The light microscopic appearance of these control kidneys served as a reference for analyzing glomerular morphology of mice given HAF.

Group Ib, five to nine week old mice with high titer antibody response

Sixteen five to nine–week–old mice divided equally between NSN and OSN animals were given the high dose antigen administration schedule. All animals were sacrificed at four weeks. All of the plasma anti-HAF levels were significantly higher at four weeks in this group than in Group Ia ($P < 0.01$) (Table 1), and equivalent to levels seen in four to eight month

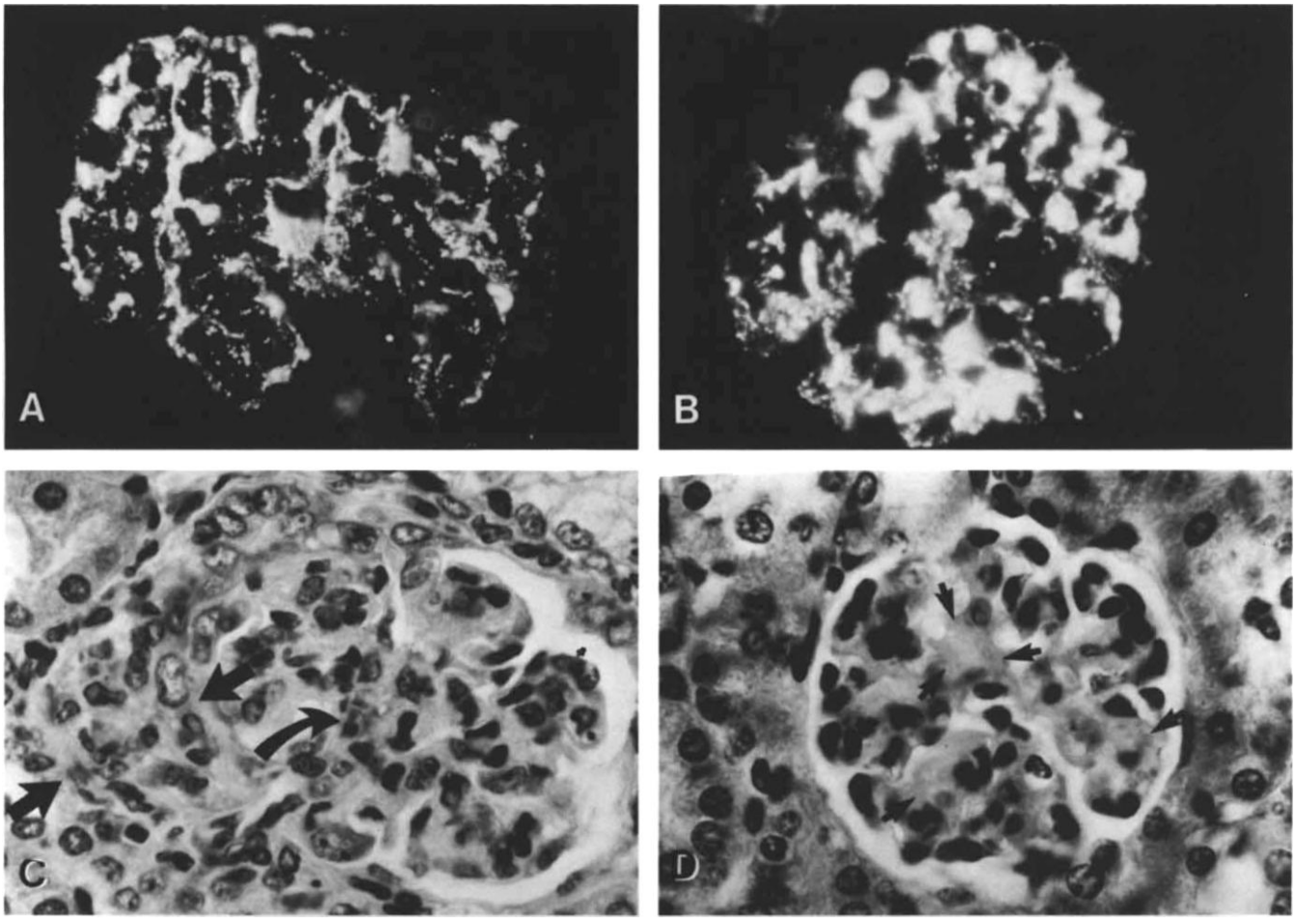

Fig. 5. *There was no immunohistochemical difference in staining for HAF in four to eight month NSN (A) and OSN (B) mice given 4 mg HAF daily for six weeks who mounted high–titered antibody responses.* However, NSN mice invariably had necrotizing and/or crescentic glomerulonephritis (**C**), whereas OSN mice glomeruli were characterized by mesangial matrix expansion (**D**). The NSN glomerulus in panel C has karyorrhexis (curved arrow) and a cellular crescent (between the two straight arrows). The OSN glomerulus in panel D has marked matrix expansion (arrows) corresponding to the massive electron dense deposits seen in Figure 7. All photomicrographs (×600), A and B anti-HAF immunofluorescence microscopy, C and D hematoxylin and eosin.

old mice (Group II mice). There was no difference in antibody levels between NSN and OSN mice ($P > 0.3$). Because of the significantly different level of immune response, Ib mice were analyzed separately from Ia mice despite the similarity of their age.

NSN and OSN mice had equivalent glomerular deposition of immunoglobulin, C3 and HAF as assessed by immunofluorescence microscopy. By light microscopy, one NSN mouse had no glomerular lesion, one had a 2+ proliferative, three had a necrotizing and three had a crescentic nephritis. Only three OSN mice had a 1+ proliferative lesion; while the other five animals had no glomerular morphologic alterations (NSN vs. OSN, $P < 0.01$). Glomerular cell counts in NSN mice revealed a mean of 53 ± 2 cells/glomerulus ($N = 8$), a greater number than that found in OSN mice who had 28 ± 2 ($N = 8$, $P < 0.001$). Thus these NSN mice with a high–titered antibody response had a proportionally greater degree of glomerular damage than Group Ia mice.

Group II, four to eight month old NSN and OSN mice

The effect of HAF administration was explored in older animals, since the level of C5 increases with age in NSN mice [14]. Four to eight month old animals were administered 4 mg HAF six days per week. At two weeks, four NSN and four OSN animals were sacrificed and had similar 3 to 4+ mesangial deposition of HAF, IgG, IgM, and C3 by immunofluorescence microscopy. Rare capillary wall immunoreactant deposition was seen. All four NSN mice had 3+ necrotizing proliferative lesions by light microscopy, whereas two OSN animals had a 2+ proliferative lesion and two had no morphologic abnormality.

Fourteen NSN and fifteen OSN mice were sacrificed at four to five weeks. Equivalent mesangial deposition of immunoglobulin, C3 and HAF was found in both groups, so that NSN and OSN animals were immunohistochemically indistinguishable (Figs. 1 and 5). Substantial capillary wall localization of HAF,

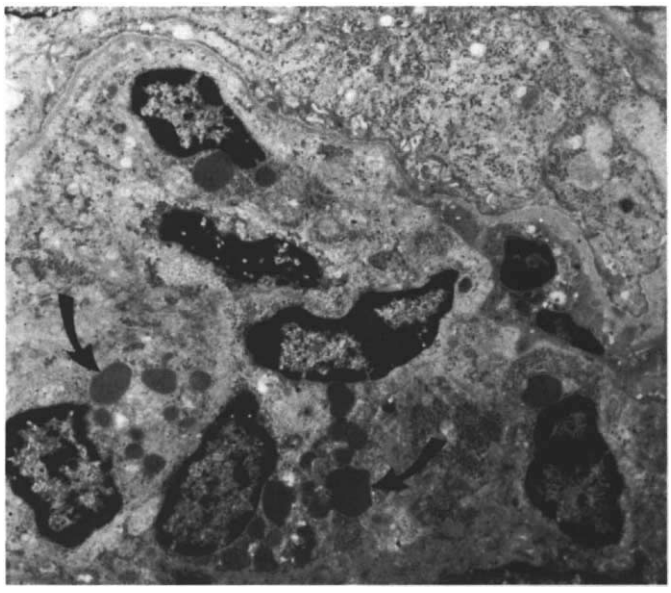

Fig. 6. *Electron micrograph of a glomerulus from a NSN mouse with proliferative glomerulonephritis showing mononuclear phagocytes with numerous cytoplasmic phagolysosomes (arrows) (×7,500).*

C3, and IgG was also observed. By light microscopy, a readily distinguishable difference between C5 sufficient and deficient animals was apparent (Figs. 3 and 5) ($P < 0.001$). All 14 NSN mice had diffuse proliferative glomerulonephritis with many areas of karyorrhexis, and 12 of 14 mice had crescent formation (Fig. 5). The cellularity of these glomeruli was due to mesangial cell proliferation as well as an influx of mononuclear leukocytes, best identified by electron microscopy (Fig. 6). Polymorphonuclear leukocyte infiltration was rare. In contrast, 10 of 15 OSN mice had glomerular alterations characterized primarily by matrix expansion rather than cellular proliferation, and had no areas of necrosis or crescent formation. Five OSN animals did have areas of necrosis, and four of these had crescentic disease. The nature of the matrix expansion seen in OSN mice by light microscopy was resolved on electron microscopy. Large dense deposits of presumed immune complex material were observed in the mesangium, subendothelial, and to a lesser degree subepithelial areas (Fig. 7). The hypercellularity of the NSN lesion was documented by the difference in mean glomerular cell counts (NSN: 61 ± 2 cells/glomerulus ($N = 14$) vs. OSN: 36 ± 2 cells/glomerulus ($N = 15$, $P < 0.001$).

In the tubulointerstitial compartment, 13 of 14 NSN mice had varying degrees of cellular infiltration, while only five of 15 OSN were similarly affected (Figs. 4 and 8). These five OSN mice were the same animals with glomerular necrosis and crescentic lesions. In no animal in any group was there evidence of extraglomerular vascular injury. Cage control NSN and OSN mice of four to eight months of age did not have glomerular or interstitial lesions.

A significant difference was observed between the mean serum creatinine of 0.51 ± 0.04 mg/dl (± SEM) in NSN mice and 0.35 ± 0.05 in OSN mice ($P < 0.05$). Moreover, when the serum creatinines were compared with a composite morphologic score (that is, glomerular plus tubulointerstitial score), a close correlation was observed between the pathologic lesion and impairment of renal function ($r = 0.82$, $P < 0.001$; Fig. 9). Additionally, the five severely affected OSN animals with composite histologic scores of 5 or greater had statistically lower creatinines than similarly affected NSN animals ($P < 0.05$).

Plasma anti-HAF levels (Table 1) were not statistically different between these older NSN and OSN mice at two or four weeks. However, older mice had significantly higher plasma antibody levels than Group Ia young mice at two and four weeks ($P < 0.01$).

Group III, five month old mice given low dose HAF

Previous investigations using this model of glomerulonephritis have demonstrated that glomerular injury is dependent on the dose of the administered antigen [10]. In view of the differences in the immunoreactant localization and light microscopic morphology of younger and older mice, we sought to establish whether a lower antigenic load, when given to older mice, would magnify NSN and OSN differences. Five–month–old mice were given 1 mg HAF on alternate days. In both NSN and OSN mice, five animals were sacrificed at two weeks and seven at four weeks. At two weeks there was a trend towards an increase in HAF, IgG, IgM, and C3 glomerular localization observed in the mesangial region of NSN when compared to OSN mice, but this difference was not statistically significant. By four weeks, however, no differences in antigen localization were discernable. At two weeks, no morphologic abnormality was seen by light microscopy in either NSN or OSN mice. At four weeks, none of the seven OSN mice had developed glomerular or interstitial alterations; whereas four of seven NSN had necrotizing lesions and two others had proliferative changes (Fig. 3). NSN glomeruli had 50 ± 2 cells/glomerulus ($N = 7$) compared to OSN glomerular cell counts of 33 ± 2 cells/glomerulus ($N = 7$, $P < 0.001$). Plasma anti-HAF levels were similar in NSN and OSN animals, with higher levels seen at two weeks than at four weeks (Table 1).

Discussion

In the present study, we evaluated the effects of C5 deficiency in a model of immune complex nephritis in which the antigen is repetitively administered for up to six weeks. The variables of animal age, antigen dose, and level of immune response were evaluated in mice deficient in C5 and their normal congenic pair. Young NSN mice with low titers of anti-HAF production developed mild proliferative glomerular lesions, while OSN animals producing similar titers of anti-HAF had no apparent morphologic abnormality. Young NSN mice who mounted a high titer antibody response developed severe proliferative, and frequently necrotizing and crescentic glomerulonephritis. Young OSN mice producing comparable amounts of anti-HAF had mild proliferative glomerular abnormalities or no lesion at all. In older mice in whom a large immune response was invariably produced, profound interstrain differences in the morphologic expression of disease were evident. NSN mice always had proliferative, necrotizing lesions, and most had crescentic nephritis. OSN glomeruli were largely affected by expansion of the mesangial matrix with immune complex material. Five of fifteen OSN mice had a more severe lesion, but their glomeruli were marked by a relatively greater increase in matrix expansion without the proliferative

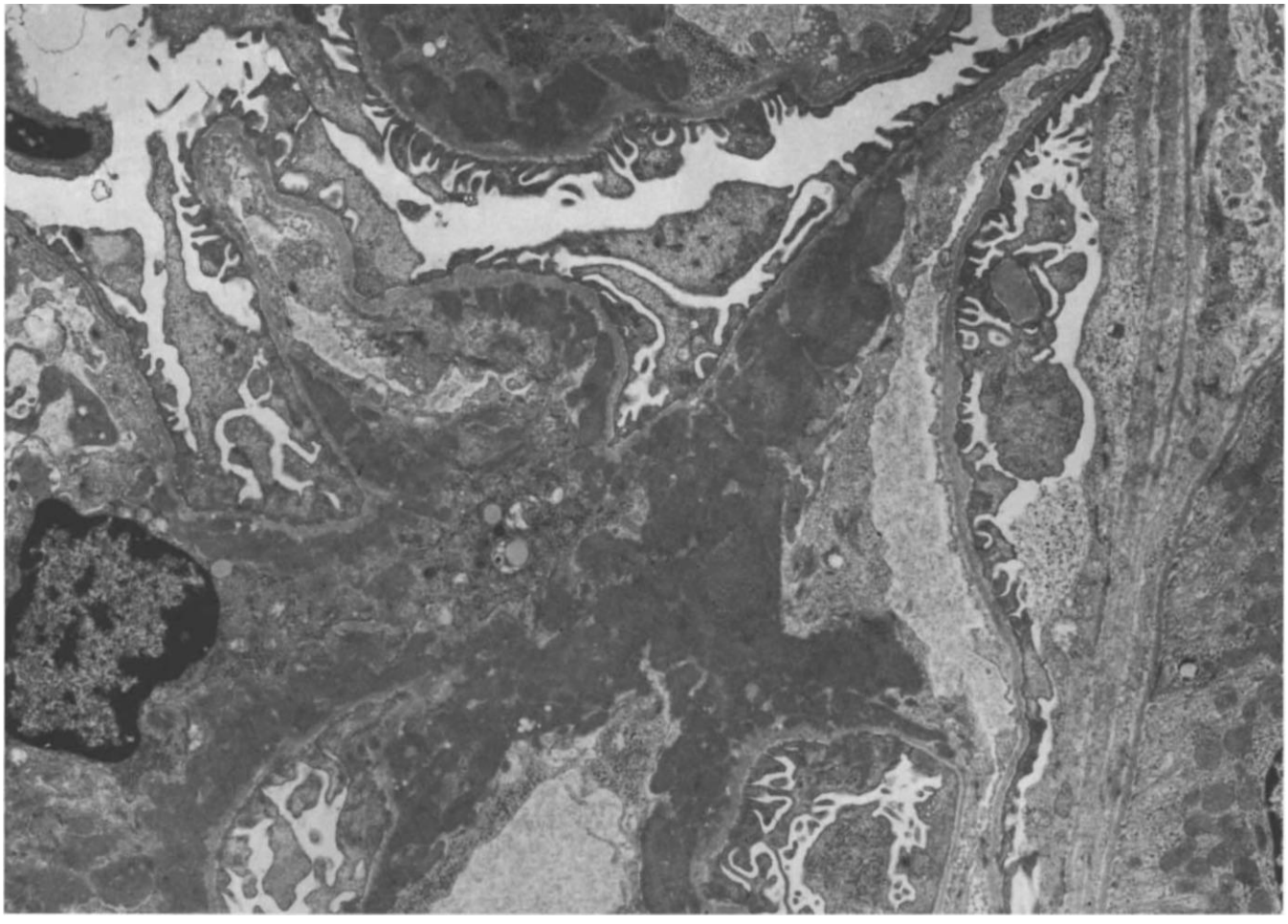

Fig. 7. *Electron micrograph of a glomerulus from a four to eight–month–old OSN given 4 mg HAF daily.* There are massive mesangial electron dense deposits that are extending into the juxtamesangial subendothelial zone. (×4,750).

response seen in NSN mice. The glomerular hypercellularity of NSN mice was due to apparent mesangial cell proliferation and mononuclear leukocyte infiltration, without the presence of polymorphonuclear leukocytes. NSN mice frequently had tubulointerstitial nephritis, while only a few OSN animals were so affected.

The morphologic expression of disease activity was correlated with impaired renal function. In Group Ia where histologic damage was minimal, serum creatinine levels were no higher than control mice. In the most severely diseased animals, Group II, serum creatinine levels in NSN mice were significantly greater than those in OSN animals. The degree of injury defined by the morphological scoring system used in this study was closely correlated with serum creatinines ($r = 0.82$).

We postulated that older mice, uniformly capable of a high titered antibody response, would have a milder lesion if given less antigen. Such milder disease would be, in some respects, analogous to the mild disease seen in those young mice with low anti-HAF production, which resulted in clear distinctions in immune complex localization between NSN and OSN mice. When five–month–old mice were given 1 mg HAF on alternate days, rather than 4 mg/day, morphologic differences in NSN and OSN mice were magnified, but no statistically significant differences in immune complex localization could be discerned. While neither NSN or OSN mice had glomerular lesions by week two, only the NSN mice had proliferative and necrotizing glomerular lesions by four weeks of immunization. The C5 deficient mouse glomeruli were histologically similar to control animals.

The presence of C5 is essential for the full expression of injury in this model of immune complex induced glomerular injury. Whether the differences between NSN and OSN mice were due to the membranolytic or leukotactic properties of complement [15] cannot be determined by our studies. However, since OSN mice are capable of complement cascade activation up to the C3 step, the glomerular lesion observed in NSN mice was not attributable to the formation of activated complement components prior to the formation of C5. This is further supported by the observation on immunofluorescence microscopy of similar amounts of C3 in glomeruli of OSN and NSN mice.

Under some conditions, C5 may play a role in immune complex localization in tissue. In mice who produced a low titer of anti-HAF, the absence of C5 affected the localization of HAF in glomeruli. OSN mice had trace amounts of HAF deposited in glomeruli, while NSN mice had substantial antigen localization

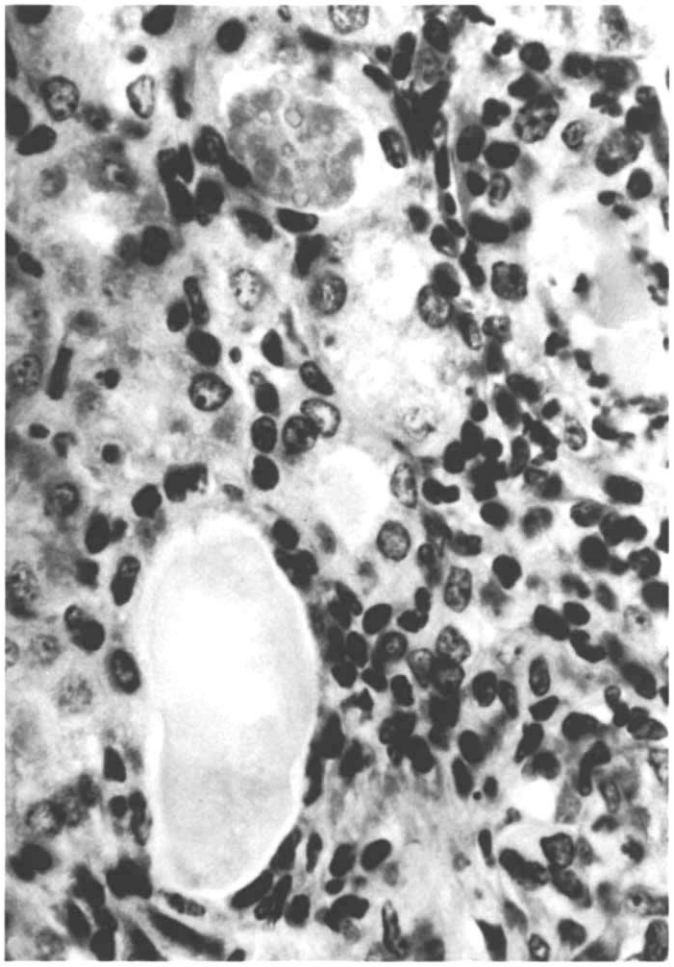

Fig. 8. *Tubulointerstitial injury, including tubular atrophy, hyaline and erythrocyte casts, and interstitial mononuclear leukocyte infiltration, in a four to eight–month–old NSN given 4 mg HAF daily for six weeks.* (×600, hematoxylin and eosin).

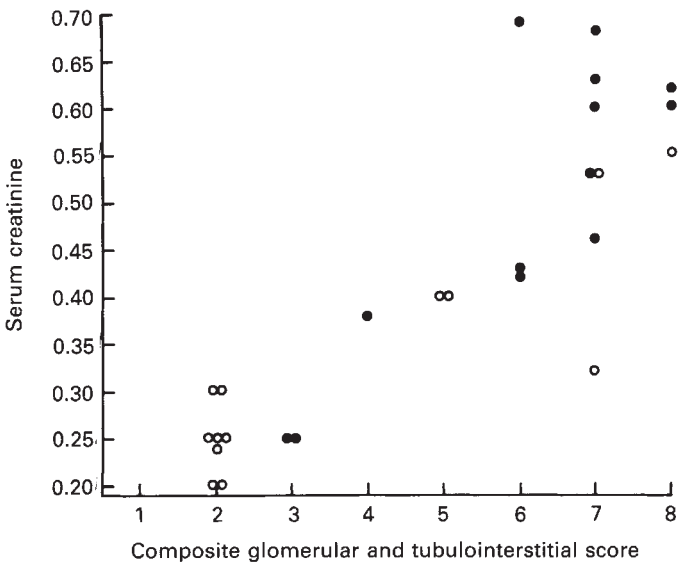

Fig. 9. *The serum creatinines were measured in Group II NSN and OSN mice.* A significantly higher level of serum creatinine was seen in NSN (●) than OSN mice (○) ($P < 0.05$). When these creatinines were compared with a composite morphologic score obtained by adding the glomerular and tubular scores, a close linear relationship was observed ($r = 0.82$).

in mesangial regions. This effect may have had a role in the development of the milder lesion observed in these young OSN mice.

In older C5-deficient mice in whom a high titered immune response was produced, large amounts of immune complex material were observed in mesangial regions on light and electron microscopy. In these mice it is possible that the build-up of immune complex material seen in OSN mice was due to the inability of C5 deficient mice to solubilize previously deposited immune complex material. Bartolotti and Peter [16] have demonstrated that decomplemented rabbits given acute immune complex nephritis have a significant decrease in their rate of clearance of glomerular bound antigen and C3. Our studies raise the possibility of a role for terminal complement components in this solubilization process. Alternatively, larger areas of glomerular acellular immune deposits in OSN mice could be due to the absence of infiltrating leukocytes and resultant diminished degradation of the deposits. The fact that a few of the Group II OSN mice did have severe glomerular lesions suggests that proximal complement components or non-complement mediators of injury must also be playing a role in this model of glomerulonephritis.

In conclusion, C5, or some other terminal complement component(s) dependent upon C5 for activation play a pivotal role in the morphologic expression of glomerular injury and impairment of renal function in this model of heterologous protein–induced murine glomerulonephritis.

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